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# Indole-7-carbaldehyde thiosemicarbazone as a flexidentate ligand toward $\text{Zn}^{\text{II}}$ , $\text{Cd}^{\text{II}}$ , $\text{Pd}^{\text{II}}$ and $\text{Pt}^{\text{II}}$ ions: cytotoxic and apoptosis-inducing properties of the $\text{Pt}^{\text{II}}$ complex.

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## Summary

A new thiosemicarbazone ( $\text{LH}_2$ ) derived from indole-7-carbaldehyde was synthesized and reacted with  $\text{Zn}^{\text{II}}$ ,  $\text{Cd}^{\text{II}}$ ,  $\text{Pd}^{\text{II}}$  and  $\text{Pt}^{\text{II}}$  salts. The reactions with zinc and cadmium salts in 2:1 (ligand/metal) molar ratio afforded complexes of the type  $\text{MX}_2(\text{LH}_2)_2$ , ( $\text{X} = \text{Cl}$ ,  $\text{Br}$  or  $\text{OAc}$ ), in which the thiosemicarbazone acts as a neutral *S*-monodentate ligand. In the presence of potassium hydroxide, the reaction of  $\text{LH}_2$  with  $\text{ZnBr}_2$  resulted in deprotonation of the thiosemicarbazone at the hydrazine and indole nitrogens to form  $\text{Zn}(\text{L})(\text{CH}_3\text{OH})$ . The reaction of  $\text{LH}_2$  with  $\text{K}_2\text{PdCl}_4$  in the presence of triethylamine, afforded  $\text{Pd}(\text{L})(\text{LH}_2)$  which contains two thiosemicarbazone ligands: one being dianionic *N,N,S*-tridentate while the other one is neutral *S*-monodentate. When  $\text{PdCl}_2(\text{PPh}_3)_2$  was used as the  $\text{Pd}^{\text{II}}$  ion source,  $\text{Pd}(\text{L})(\text{PPh}_3)$  was obtained. In a similar manner, the analogous platinum complex,  $\text{Pt}(\text{L})(\text{PPh}_3)$ , was synthesized. The thiosemicarbazone in the latter two complexes behaves in a dianionic *N,N,S*-tridentate fashion. The platinum complex was found to have significant cytotoxicity toward four cancer cells lines, namely MDA-MB-231, MCF-7, HT-29, and HCT-116 but not toward the normal liver WRL-68 cell line. The

apoptosis-inducing properties of the Pt complex was explored through fluorescence microscopy visualization, DNA fragmentation analysis and propidium iodide flow cytometry.

## Introduction

Thiosemicarbazones constitute a class of ligands that have attracted a great deal of attention owing to their versatile coordination behavior toward metal ions.<sup>1</sup> Figure 1 presents a variety of coordination modes observed in thiosemicarbazone metal complexes. The coordination usually occurs through the sulfur atom and either the azomethine N atom to create a five-membered chelate ring (**1**),<sup>2</sup> or the hydrazine N atom to make a more strained four membered ring (**2**).<sup>3,4</sup> If the ligand contains a third donor site (D) appropriately located for coordination (*e.g.* salicylaldehyde thiosemicarbazone), a *D,N,S*-tricoordination normally takes place (**3**);<sup>5-9</sup> however examples are found in literature where the potential O donor atom from a salicylaldehyde thiosemicarbazone does not bind to the metal and the ligand shows an *N,S*-bidentate mode (**4** and **5**).<sup>9-11</sup> In addition, the *S*-donor atoms of thiosemicarbazones can bridge metal ions to form dinuclear,<sup>12,13</sup> or multinuclear metal complexes (**6**).<sup>14-16</sup> Other coordination modes observed in thiosemicarbazones metal complexes include *S*-monodentate (**7**),<sup>17-19</sup> and *N*(thioamide),*S*-bidentate (**8**).<sup>20</sup> The applications of these metal complexes range from biological to material sciences fields. Examples are cytotoxic, antibacterial and antimalarial activities of thiosemicarbazone Pd<sup>II</sup> and Pt<sup>II</sup> complexes,<sup>5-8,21</sup> and non-linear optical properties associated with thiosemicarbazone Zn<sup>II</sup> and Cd<sup>II</sup> complexes.<sup>17-19</sup>

The present paper introduces a new thiosemicarbazone ligand (LH<sub>2</sub>, Fig. 1) derived from indole-7-carbaldehyde. The structure of the molecule mimics that of salicylaldehyde thiosemicarbazone: both thiosemicarbazones have the third potential donor atom (D) linked to the carbonylic carbon *via* two intervening atoms; however, the third donor in LH<sub>2</sub> is the softer N atom vs. the harder O

atom in the salicylaldehyde derivative (**4** and **5**). The complexation behavior of LH<sub>2</sub> toward Zn<sup>II</sup>, Cd<sup>II</sup>, Pd<sup>II</sup> and Pt<sup>II</sup> is studied. Furthermore, the synthesized Pt<sup>II</sup> complex was evaluated for its cytotoxicity and apoptosis-inducing properties.

## Results and discussion

The condensation reaction of indole-7-carboxaldehyde with thiosemicarbazide resulted in the formation of the corresponding thiosemicarbazone, LH<sub>2</sub>. Similar to most thiosemicarbazones, the predominant form of LH<sub>2</sub> in both the solid state and solution is the thione tautomer (rather than the thiol tautomer). It is indicated by the absence of S-H band in the IR spectrum (at *ca.* 2700 cm<sup>-1</sup>) and any signal attributable to the thiol proton in the <sup>1</sup>H NMR spectrum. The NNH signal of the thione is observed at  $\delta$  = 11.25 ppm.

The synthesized thiosemicarbazone, LH<sub>2</sub>, was reacted with Zn, Cd, Pd and Pt salts under various conditions to afford the corresponding metal complexes in moderate to high yield (Scheme 1). Upon reaction of LH<sub>2</sub> with MX<sub>2</sub> (M = Zn or Cd; X = Cl, Br or OAc) in a ligand/metal ratio of 2:1, metal complexes with the formula of MX<sub>2</sub>(LH<sub>2</sub>)<sub>2</sub> were obtained. As revealed by X-ray crystallographic analysis, in the solid state structures of these complexes, LH<sub>2</sub> acts as a neutral *S*-monodentate ligand; however, the solution <sup>1</sup>H and <sup>13</sup>C NMR signals of the compounds do not show any shift from those of the free ligand suggesting that decomposition into the free ligand and metal salts in DMSO-*d*<sub>6</sub> solution occurs. In contrast, the reaction of LH<sub>2</sub> with ZnBr<sub>2</sub> in a 1:1 ratio and in the presence of KOH afforded a complex with the formula of Zn(L)(CH<sub>3</sub>OH). The <sup>1</sup>H NMR spectrum of this complex confirms the deprotonation of the indole NH and thioamide NNH of the ligand while its <sup>13</sup>C NMR spectrum shows an upfield shift of the CS signal (~ 4 ppm) and a downfield shift of the azomethine CHN signal (~ 4 ppm) from those in the spectrum

of LH<sub>2</sub>. Attempts to grow an X-ray quality crystal from this compound failed; however, based on elemental analysis and NMR spectroscopy the ligand in this compound is believed to act as a dianionic tridentate chelate, coordinating the metal atom through its sulfur, azomethine N and indole N atoms.

The reaction of LH<sub>2</sub> with K<sub>2</sub>PdCl<sub>4</sub> in a 2:1 (ligand/metal) ratio and in the presence of triethylamine, led to the formation of Pd(L)(H<sub>2</sub>L). In agreement with the X-ray crystal structure, the solution <sup>1</sup>H and <sup>13</sup>C NMR spectra of this complex clearly show the existence of two types of the thiosemicarbazone ligand in the structure, one being doubly deprotonated while the other one is neutral. The upfield shift (5-6 ppm) of the two CS signals in the <sup>13</sup>C NMR spectrum of the complex compared to that of the free LH<sub>2</sub> suggests that the S atoms of both ligands remains coordinated to the Pd<sup>II</sup> center in the DMSO-*d*<sub>6</sub> solution.

The reaction of LH<sub>2</sub> with PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> in a 1:1 ratio and in the presence of triethylamine, yielded Pd(L)(PPh<sub>3</sub>). The platinum analog, Pt(L)(PPh<sub>3</sub>), was similarly obtained. The IR spectra of the two complexes closely resemble each other and agree with reported peaks for coordinated PPh<sub>3</sub> (e.g., 745, 691 and 537 for the Pd complex.<sup>6</sup> The solution <sup>1</sup>H and <sup>13</sup>C NMR spectra of the Pd complex do not show any characterizable species, suggesting the instability of the compound in DMSO solution. In contrast, the 1D- and 2D-NMR spectra of the Pt complex in DMSO-*d*<sub>6</sub> is in accordance with the solid state structure. In the <sup>1</sup>H NMR spectrum of the platinum complex the azomethine CHN signal ( $\delta$  = 8.80 ppm) appears as a doublet due to the coupling with the phosphorus atom.<sup>5,10</sup> The phosphorus atom is also coupled with some of the carbon atoms so that their signals appear as doublets in the <sup>13</sup>C NMR spectrum. It is worth mentioning that the NMR solution of the Pt complex, kept at room temperature for a month, gave the same spectra as before, indicating the stability of the complex in DMSO solution.

**Crystal structure of  $\text{ZnBr}_2(\text{LH}_2)_2 \cdot 2\text{MeOH}$ .** The molecular structure of  $\text{ZnBr}_2(\text{LH}_2)_2$  is shown in Fig. 2 and the selected bond lengths and angles are listed in Table S1†. Two thiosemicarbazone molecules, acting as neutral monodentate ligands, coordinate the metal center through their sulfur atoms. The two ligands are almost planar with r.m.s. deviation from the planes of S1 and S2 containing ligands being 0.0503 and 0.0284 Å respectively; the dihedral angle between the planes is 60.92(7)°. Two Br atoms complete the distorted tetrahedral geometry around the Zn atom with coordination angles in the range of 100.26(3)–113.80(2)°. The Zn-S bond lengths are comparable to those observed in similar structures,<sup>17,19,22</sup> and so are the Zn-Br distances.<sup>19</sup> There is intramolecular hydrogen bonding between hydrogens bonded to N3 and N7 and the Br ligands (Table S3†). Moreover, intermolecular N-H...Br interactions occur in the crystal to connect the adjacent molecules into chains along the *a* axis. The crystal structure contains methanol solvent molecules which are H-bonded to these chains.

**Crystal structure of  $\text{CdCl}_2(\text{LH}_2)_2 \cdot \text{MeOH}$ .** The molecular structure is depicted in Fig. 3 and Table S1† lists the selected bond lengths and angles. The structure is similar to that of the Zn complex. The thiosemicarbazone molecules are nearly planar (r.m.s. deviation = 0.0236 and 0.0715 Å for S1 and S2 containing ligands, respectively) and make a dihedral angle of 80.97(6)°. The Cd-S distances are compatible with those in similar structures.<sup>18,23</sup> Unlike the zinc complex structure, the intramolecular H-bonding occurs between the thioamide hydrogens, N4 and N8, and the halide ligands (Table S3†). The crystal packing shows interdigitating layers in the *bc* plane formed by intermolecular N-H...Cl interactions. Disordered solvent methanol molecules are N-H...O bonded to these layers.

**Crystal structure of  $\text{Cd}(\text{OAc})_2(\text{LH}_2)_2 \cdot 2\text{MeOH}$ .** The asymmetric unit of the crystal structure consists of two crystallographically independent Cd complexes and four methanol molecules. The two complexes have only slightly different geometrical parameters; the weighted r.m.s. fit for the superposition of the non-H atoms in both molecules is 0.103 Å. The structure of one of the complex molecules is depicted in Fig. 4 and selected bond lengths and angles are listed in Table S1†. The  $\text{Cd}^{\text{II}}$  atom is coordinated by two thiosemicarbazone molecules which behave as neutral S-donor monodentate ligands. One monodentate and one bidentate acetate groups complete a five-coordinate metal complex. The coordination geometry can be determined using the index  $\tau = (\beta - \alpha)/60$  where  $\beta$  is the largest angle and  $\alpha$  is the second one around the metal center.<sup>24</sup> For an ideal square pyramidal geometry  $\tau = 0$ , while it is 1 for a perfect trigonal bipyramidal geometry. In the present structure  $\tau$  computes to 0.23 which indicates a distorted square-pyramidal geometry. The r.m.s deviation from planarity for the S1 and S2 containing ligands are 0.068 and 0.095 Å, respectively, and the two planes make a dihedral angle of 4.96(12)°. There is intramolecular hydrogen bonding between thioamide nitrogens N4 and N8 and the monodentate acetate ligand (Table S3†). In the crystal, the complexes are connected through intermolecular N-H...O and N-H...N interactions to form infinite chains along the crystallographic *a* direction. The methanol solvate molecules are H-bonded to the formed chains.

**Crystal structure of  $\text{Pd}(\text{L})(\text{HL}_2)$ .** The crystal structure of  $\text{Pd}(\text{L})(\text{HL}_2)$  is depicted in Fig. 5. The Pd center is coordinated by two different thiosemicarbazone ligands, one of them being dianionic *N,N',S*-tridentate, while the other one acts as a neutral *S*-monodentate ligand. The two ligands are almost planar with r.m.s. deviation from planarity for their non-H atoms of 0.087 and 0.034 Å for the tridentate and monodentate ligands respectively. The two planes make a dihedral

angle of  $77.67(3)^\circ$ . The selected bond lengths and angles for the structure are gathered in Table S2†. The longer S1-C10 bond length than S2-C20 distance points out the higher thiolic character of the tridentate ligand compared to the monodentate ligand. The two ligands differ also in their configuration about N3-C10 (*Z*) and N8-C20 (*E*) bonds, in keeping with what was observed in the structures of similar Pd complexes.<sup>25,26</sup> The metal atom is in a distorted square-planar geometry and is displaced by  $0.0575(6)$  Å out of the coordination plane (N1/N2/S1/S2). In the crystal, pairs of the metal complex molecules are doubly connected through N8-H8A...N3 hydrogen bonding into centro-symmetric dimers (Table S3†). The resulting dimers are connected to each other *via* C-H... $\pi$  and N-H... $\pi$  interactions to form a three-dimensional network with voids of the size of  $331 \text{ \AA}^3$  wherein disorder solvent molecules reside.

**Crystal structure of Pd(L)(PPh<sub>3</sub>)•DMF.** The molecular structure of Pd(L)(PPh<sub>3</sub>) and the labeling scheme is depicted in Fig. 6. The doubly deprotonated thiosemicarbazone ligand coordinates the Pd atom in an *N,N',S*-tridentate manner to create five- and six-membered chelate rings. A triphenylphosphine group occupies the fourth coordination site, making a square-planar palladium complex with the metal atom being  $0.0236$  Å out of the coordination plane, N1/N2/S1/P1. The ligand is almost planar with an r.m.s. deviation of  $0.0536$  Å. Table S2† lists the selected bond lengths and angles for the structure. While the Pd-N1 and Pd-S distances are comparable to those in Pd(L)(LH<sub>2</sub>), the Pd-N2 distance in the present structure is  $0.0431$  Å longer than that in the former structure. This can be attributed to the *trans*-influence of the PPh<sub>3</sub> ligand as was observed in similar palladium complex structures.<sup>7,8</sup> In the crystal, pairs of the metal complex molecule are connected into centro-symmetric dimers through N4-H4B...N3 hydrogen bonds and these are linked *via* C-H... $\pi$  interactions to form a three-dimensional



polymeric structure (Table S3†). In the crystal, disordered DMF solvate molecules exist as hydrogen bond acceptors in N4-H4A...O interactions.

**Crystal structure of Pt(L)(PPh<sub>3</sub>)•0.75MeOH.** The molecular structure of the platinum(II) complex (Fig. 7) resembles that of Pd(L)(PPh<sub>3</sub>). The planar thiosemicarbazone (r.m.s deviation = 0.0812 Å) is doubly deprotonated and coordinated to the platinum atom through its indolic N, azomethine N and thioamide S atoms to form a five- and a six-membered chelate ring. A triphenylphosphine ligand at the fourth coordination site completes a distorted square-planar Pt<sup>II</sup> complex; the metal center is displaced from the coordination plane, N1/N2/S1/P1, by 0.0578 Å. Table S2† compiles the selected bond lengths and angles for the structure. The Pt-P is slightly shorter and Pt-N2 slightly longer than corresponding bond lengths in similar structures.<sup>27,28</sup> In the crystal, the molecules are connected through C-H... $\pi$  interactions to form hexagonal channels along the *c*-axis (void size = 264 Å<sup>3</sup>) within which there is no evidence for included solvent (Fig. 8). The outer-shell of the channel is solvated by the partially occupying methanol molecules (Table S3†).

### Cytotoxicity assay

Preliminary cytotoxicity screening for LH<sub>2</sub> and its Pd and Pt complexes against four cancer cell lines, revealed significant activity for Pt(L)PPh<sub>3</sub>. Table 1 lists the IC<sub>50</sub> values of the platinum complex after treating the cell lines for 24, 48 and 72 h. As shown by the results, for 24 h treatments the IC<sub>50</sub> values lie between 2.08-22.97  $\mu$ M. These values dropped when the treatments were prolonged, thus the IC<sub>50</sub> values for 48 h treatments range from 1.49 to 3.34  $\mu$ M

and for 72 h treatments are between 0.77-2.77  $\mu\text{M}$ . The effect of the platinum complex on normal liver cell lines (WRL-68) was also examined. As shown in Fig. 9, minimal toxicity was observed at the concentrations below 40  $\mu\text{M}$ , attesting to cancer-selective cytotoxicity of  $\text{Pt(L)(PPh}_3\text{)}$  at low doses. For the sake of comparison, cells were also treated with cisplatin, a clinical anticancer drug. As presented in Table 1, compared to cisplatin,  $\text{Pt(L)(PPh}_3\text{)}$  exerted higher or comparable cytotoxicity against the cancer cell lines and higher selectivity for the cancer cells over normal cells.

**Table 1** IC<sub>50</sub> ( $\mu\text{M}$ ) values  $\pm$  SEM of  $\text{Pt(L)(PPh}_3\text{)}$  and cisplatin on different cancer cell lines

		24 hours	48 hours	72 hours
MDA-MB-231	$\text{Pt(L)(PPh}_3\text{)}$	$22.97 \pm 4.13$	$3.34 \pm 0.18$	$2.27 \pm 0.28$
	Cisplatin	$18.09 \pm 1.32$	$16.37 \pm 0.65$	$15.64 \pm 0.77$
MCF-7	$\text{Pt(L)(PPh}_3\text{)}$	$2.23 \pm 0.13$	$1.49 \pm 0.04$	$0.77 \pm 0.045$
	Cisplatin	$14.76 \pm 0.58$	$11.89 \pm 0.71$	$9.13 \pm 0.62$
HT-29	$\text{Pt(L)(PPh}_3\text{)}$	$2.08 \pm 0.12$	$1.74 \pm 0.29$	$0.94 \pm 0.02$
	Cisplatin	$72.68 \pm 0.92$	$36.71 \pm 3.48$	$5.76 \pm 1.49$
HCT-116	$\text{Pt(L)(PPh}_3\text{)}$	$3.71 \pm 0.10$	$2.41 \pm 0.24$	$1.52 \pm 0.04$
	Cisplatin	$38.73 \pm 8.23$	$23.95 \pm 5.18$	$5.76 \pm 1.49$
WRL-68	$\text{Pt(L)(PPh}_3\text{)}$	$52.58 \pm 0.71$	$48.01 \pm 0.49$	$48.53 \pm 0.67$
	Cisplatin	$> 80$	$13.58 \pm 2.34$	$10.32 \pm 1.62$

## Apoptosis assay

Apoptosis induction is an anti-proliferative mechanism by which the cancer cells undergo programmed death. Cells undergoing apoptosis are characterized by morphological and biochemical changes including cell shrinkage, chromatin condensation and DNA fragmentation. Based on the results of the *in vitro* cytotoxicity assay for Pt(L)PPh<sub>3</sub>, it was considered worthwhile to explore the apoptotic properties of the compound. Apoptosis in the four cancer cell lines was examined through fluorescence microscopy visualization, DNA fragmentation analysis and propidium iodide flow cytometry.

**Fluorescence microscopy visualization.** Apoptotic cells exhibit increased plasma membrane permeability to certain fluorescent dyes. Acridine orange (AO)/propidium iodide (PI) is a fluorochrome mixture for nuclear staining which enables distinction between viable, apoptotic and necrotic cells. In this work, the four cancer cell lines were subjected to AO/PI staining after treatment with respective IC<sub>50</sub> concentrations of Pt(L)PPh<sub>3</sub> for 48 h. Fluorescence microscopy reveals that in all the cancer lines the platinum complex induced cell death mainly *via* apoptosis (Fig. 10).

Fluorochrome-labelled Annexin V staining is another way for microscopic visualization of the apoptotic cells. Normal cells have the phospholipid phosphatidylserine (PS) on their inner-leaflet of the membranes, but upon undergoing apoptosis, PS becomes exposed on the external side of the cellular membrane. Annexin V binds to cell membranes containing exposed PS, thus labelled annexin V can be used to detect apoptotic cells at early stages. In this study FITC-annexin V was applied to target the apoptotic cells after treatment of the cancer cells with IC<sub>50</sub> concentrations of Pt(L)PPh<sub>3</sub> for 48 h. The fluorescence microscopic examination of the treated

cells when compared to those grown under the control conditions attest to the binding of FITC-annexin V to the externalized PS in the treated cells, thus induction of apoptosis by the platinum complex in the four cancer cell lines (Fig. 11).

**DNA Fragmentation analysis.** The degradation of nuclear DNA into nucleosomal units is a biochemical feature of apoptosis. To evaluate the apoptotic DNA fragmentation in the cancer cells, the four cell lines were incubated with the corresponding  $IC_{50}$  values of Pt(L)PPh<sub>3</sub> for 48 h. The cells were then lysed, the nuclear DNA was extracted, and subjected to gel electrophoresis. Inspection of the electrophoretic profiles revealed a ladder formation (fragments range from 400 to 1000 bp), attesting to the occurrence of apoptosis (Fig. 12).

**Cell cycle analysis.** The DNA content of cells duplicates during the S phase, therefore cells in the G<sub>0</sub> and G<sub>1</sub> phases (before the S phase) have unreplicated DNA, while those in the G<sub>2</sub> and M phases (after the S phase) have replicated DNA. Analysis of cell cycles by flow cytometry enables us to distinguish and quantify the cells in different phases of the cell cycle. On the flow cytometry DNA histograms cells with degraded and thus hypodiploid DNA are represented in so-called “sub-G<sub>0</sub>/G<sub>1</sub>” peaks therefore, sub-G<sub>0</sub>/G<sub>1</sub> is a specific marker of cell death by apoptosis.<sup>28</sup> Flow cytometry analysis of the four cancer cell lines after treatments with respective  $IC_{50}$  concentrations of the Pt(L)PPh<sub>3</sub> for 24 and 48 h was carried out. The DNA histograms (Fig. 13) suggest that the HCT-116 and MDA-MB-231 cell cycles were arrested in the G<sub>0</sub>/G<sub>1</sub> phase whereas the HT-29 and MCF-7 cell cycle arrests happened in the S phase. Moreover, the histogram shows gradual increases in the populations of sub-G<sub>0</sub>/G<sub>1</sub> cells during the incubation

periods. Overall flow cytometry suggests the induction of apoptosis by the platinum complex in the cancer cells through G<sub>0</sub>/G<sub>1</sub> or S phase cell cycle arrest.

## Conclusion

LH<sub>2</sub>, a new thiosemicarbazone whose structure resembles that of salicylaldehyde thiosemicarbazone, is a polydentate ligand having flexidentate character toward metal ions. In the examples in this study it exhibits two coordination modes, *i.e.* *S*-monodentate (in its neutral form) and *N,N,S*-tridentate (in a dianionic form). The latter creates a five- and a six-membered chelate ring with the metal ion. It would appear that the coordination mode is ruled by the basicity of the reaction medium, ligand/metal ratio and the identity of the metal salt. The indole N atom is either deprotonated and coordinated to the metal center or is intramolecularly H-bonded to the azomethine N atom. In all the examples, regardless of the charge and denticity of the ligand and the coordination geometry of the complex, the thiosemicarbazone ligands keep their planarity.

The platinum complex introduced in this article has shown a remarkable and cancer-selective cytotoxicity against a variety of cell lines as inferred from the MTT-based IC<sub>50</sub> values and different apoptotic assay methods thus, can be considered as a potential anticancer drug.

## Experimental

### Materials and Measurements

Indole-7-carbaldehyde was purchased from Sigma-Aldrich Company. Ethanol was distilled prior to use. The IR spectra were obtained using a Perkin–Elmer Spectrum 400 ATR-FTIR

spectrometer. The NMR spectra were recorded with a JEOL Lambda 400 MHz FT-NMR spectrometer.

## Synthesis

**LH<sub>2</sub>.** A mixture of indole-7-carbaldehyde (1.45 g, 10 mmol) and thiosemicarbazide (0.91 g, 10 mmol) in ethanol (25 mL) containing a few drops of acetic acid was refluxed for 3 h. The solution was then partially evaporated followed by addition of distilled water to precipitate the yellowish product. It was filtered, washed with 30% aqueous ethanol and dried over silica gel. Yield: 1.9 g, 87%. Anal. Calcd for C<sub>10</sub>H<sub>10</sub>N<sub>4</sub>S: C, 55.02; H, 4.62; N, 25.67; S, 14.69. Found: C, 55.13; H, 4.45; N, 25.33%. IR (ATR): 3445, 3407, 3383, 3234, 3145, 1610, 1526, 1363, 1335, 1290, 944, 897, 825, 795, 728, 712 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  6.53 (dd, 1H, Ar-*H*); 7.06 (t, 1H, Ar-*H*); 7.17 (d, 1H, Ar-*H*); 7.37 (t, 1H, Ar-*H*); 7.65 (d, 1H, Ar-*H*); 8.25 (s, 1H, NH<sub>2</sub>); 8.28 (s, 1H, NH<sub>2</sub>); 8.33 (s, 1H, CHN); 10.98 (s, 1H, indole NH); 11.25 (s, 1H, NNH) ppm. <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  102.42, 117.59, 119.63, 123.60, 126.19, 127.03, 129.23, 131.17 (*Ar*); 146.47 (CHN); 177.64 (CS) ppm.

**ZnBr<sub>2</sub>(LH<sub>2</sub>)<sub>2</sub>.** A solution of ZnBr<sub>2</sub>·2H<sub>2</sub>O (0.13 g, 0.5 mmol) in methanol (5 mL) was added to a solution of LH<sub>2</sub> (0.218 g, 1 mmol) in the same solvent (10 mL). The mixture was refluxed for 4 h and then set aside at room temperature for 5 days whereupon crystals of ZnBr<sub>2</sub>(LH<sub>2</sub>)<sub>2</sub>·2MeOH were formed. After the structure determination by X-ray crystallography, the crystals were ground and dried at 50 °C for the other analysis purposes. Yield: 0.225 g, 68%. Anal. Calcd. For C<sub>20</sub>H<sub>20</sub>Br<sub>2</sub>N<sub>8</sub>S<sub>2</sub>Zn: C, 36.30; H, 3.05; N, 16.93. Found: C, 36.69; H, 3.13; N, 16.77%. IR (ATR): 3441, 3329, 3227, 1603, 1546, 1375, 1337, 1284, 950, 814, 792, 725, 717 cm<sup>-1</sup>.

**CdCl<sub>2</sub>(LH<sub>2</sub>)<sub>2</sub>.** A solution of CdCl<sub>2</sub>·2H<sub>2</sub>O (0.11 g, 0.5 mmol) in methanol (5 mL) was added to a methanolic solution (10 mL) of LH<sub>2</sub> (0.218 g, 1 mmol). The mixture was refluxed for 4 h, then set aside at room temperature for one day whereupon X-ray quality crystals of CdCl<sub>2</sub>(LH<sub>2</sub>)<sub>2</sub>·MeOH were formed. After the structure determination, the crystals were ground and dried at 50 °C. Yield: 0.216 g, 79%. Anal. Calcd. For C<sub>20</sub>H<sub>20</sub>CdCl<sub>2</sub>N<sub>8</sub>S<sub>2</sub>: C, 38.75; H, 3.25; N, 18.08. Found: C, 38.57; H, 3.36; N, 17.98%. IR (ATR): 3399, 3285, 3187, 1605, 1549, 1371, 1337, 1287, 953, 893, 815, 797, 731, 715 cm<sup>-1</sup>.

**Cd(OAc)<sub>2</sub>(LH<sub>2</sub>)<sub>2</sub>.** A solution of Cd(OAc)<sub>2</sub>·2H<sub>2</sub>O (0.133 g, 0.5 mmol) in methanol (10 mL) was added to a solution of H<sub>2</sub>L (0.218 g, 1 mmol) in the same solvent (10 mL). The mixture was refluxed for 4 h and then set aside at room temperature overnight whereupon X-ray quality crystals of Cd(LH<sub>2</sub>)<sub>2</sub>(OAc)<sub>2</sub>·2MeOH were obtained. After the crystal structure determination, the crystals were ground and dried at 50 °C and used for further analysis. Yield: 0.169 g, 51%. Anal. Calcd. For C<sub>24</sub>H<sub>26</sub>CdN<sub>8</sub>O<sub>4</sub>S<sub>2</sub>: C, 43.21; H, 3.93; N, 16.80. Found: C, 42.66; H, 4.66; N, 16.54%. IR (ATR): 3415, 3265, 3159, 1610, 1542, 1372, 1339, 1294, 829, 793, 730, 693 cm<sup>-1</sup>.

**Zn(L)(CH<sub>3</sub>OH).** To a solution of LH<sub>2</sub> (0.218 g, 1 mmol) and KOH (0.14 g, 2.5 mmol) in methanol (10 mL), a solution of ZnBr<sub>2</sub>·2H<sub>2</sub>O (0.261 g, 1 mmol) in the same solvent (10 mL) was added and the mixture was stirred at room temperature for 24 h. The resulting precipitate was filtered, washed with 50% aqueous ethanol and dried over silica gel. Yield: 0.23 g, 74%. Anal. Calcd. For C<sub>20</sub>H<sub>20</sub>Br<sub>2</sub>N<sub>8</sub>S<sub>2</sub>Zn: C, 42.12; H, 3.86; N, 17.86. Found: C, 42.13; H, 3.42; N, 18.05%. IR (ATR): 3424, 3252, 3142, 1604, 1538, 1372, 1330, 1280, 896, 824, 795, 734 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 3.13 (d, 3H, CH<sub>3</sub>OH); 4.12 (q, 1H, CH<sub>3</sub>OH); 6.29 (s, 2H, NH<sub>2</sub>); 6.37 (d, 1H, Ar-*H*); 6.83 (t, 1H, Ar-*H*); 7.03 (d, 1H, Ar-*H*); 7.49 (d, 1H, Ar-*H*); 7.51 (d, 1H, Ar-*H*); 8.40 (s, 1H,

CHN) ppm.  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ):  $\delta$  49.13 ( $\text{CH}_3\text{OH}$ ); 100.74, 116.52, 119.15, 122.16, 123.72, 131.53, 137.74, 140.61 (*Ar*); 150.22 (*CHN*); 173.23 (*CS*) ppm.

**Pd(L)(H<sub>2</sub>L).** A solution of  $\text{K}_2\text{PdCl}_4$  (0.163 g, 0.5 mmol) in methanol (5 mL) was added to a solution of  $\text{LH}_2$  (0.218 g, 1 mmol) in the same solvent (10 mL) followed by addition of a few drops of triethylamine. The mixture was refluxed for 5 h, and then set aside at room temperature for one day whereupon brownish crystals of the product were formed. X-ray crystallographic analysis of the crystals showed inclusion of disordered solvent molecules in the crystal, therefore for the other analysis purposes the crystals were ground and dried at 50 °C. Yield: 0.194 g, 72%. Anal. Calcd. For  $\text{C}_{20}\text{H}_{18}\text{N}_8\text{PdS}_2$ : C, 44.41; H, 3.35; N, 20.71. Found: C, 44.41; H, 3.69; N, 20.32%. IR (ATR): 3420 (sh), 3309, 1600, 1550, 1506, 1304, 1231, 793, 732  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{DMSO-}d_6$ ):  $\delta$  6.48 (d, 1H, *Ar-H*); 6.57 (dd, 1H, *Ar-H*); 6.62 (br, 2H,  $\text{NH}_2$ ); 6.95 (t, 1H, *Ar-H*); 7.10 (t, 1H, *Ar-H*); 7.30 (d, 1H, *Ar-H*); 7.38 (d, 1H, *Ar-H*); 7.43 (m, 1H, *Ar-H*); 7.73 (m, 3H, *Ar-H*); 8.38 (s, 1H, *CHN*); 8.53 (s, 1H, *CHN*); 9.04 (s, 1H,  $\text{NH}_2$ ); 9.31 (s, 1H,  $\text{NH}_2$ ); 11.14 (s, 1H, indole *NH*); 12.18 (s, 1H, *NNH*) ppm.  $^{13}\text{C}$  NMR ( $\text{DMSO-}d_6$ ):  $\delta$  101.69, 102.69, 116.59, 116.83, 117.59, 119.74, 124.82, 124.89, 125.89, 127.21, 127.40, 129.34, 129.93, 131.30, 133.45, 135.29 (*Ar*); 147.52, 151.63 (*CHN*); 171.68, 172.92 (*CS*) ppm.

**Pd(L)(PPh<sub>3</sub>).** A solution of *trans*- $\text{PdCl}_2(\text{PPh}_3)_2$  (0.702 g, 1 mmol) in methanol (10 mL) was added to a solution of  $\text{LH}_2$  (0.218 g, 1 mmol) in the same solvent (10 mL) followed by addition of a few drops of triethylamine. The mixture was refluxed for 5 h and then set aside at room temperature for a few days whereupon the orange crystals of the product appeared. The crystals were ground and dried at 50 °C. Yield: 0.480 g, 82%. Anal. Calcd. For  $\text{C}_{28}\text{H}_{23}\text{N}_4\text{PPdS}$ : C, 57.49; H, 3.96; N, 9.58. Found: C, 57.23; H, 4.04; N, 9.27. %. IR (ATR): 3442 (w), 3320 (w), 1603, 1556, 1517, 1435, 1306, 1233, 792, 745, 691, 537  $\text{cm}^{-1}$ .



X-ray quality crystals of Pd(L)(PPh<sub>3</sub>).DMF were obtained from a DMF solution of the complex on standing for four days at room temperature.

**Pt(L)(PPh<sub>3</sub>).** A solution of *cis*-PtCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> (0.791 g, 1 mmol) in methanol (10 mL) was added to a solution of LH<sub>2</sub> (0.218 g, 1 mmol) in the same solvent (10 mL) followed by addition of a few drops of triethylamine. The mixture was refluxed for 6 h and then set aside at room temperature overnight whereupon X-ray quality crystals of the product were obtained. After the crystal structure determination, the crystals were washed with 50% aqueous ethanol, ground and dried at 50 °C. Yield: 0.512 g, 76%. Anal. Calcd. For C<sub>28</sub>H<sub>23</sub>N<sub>4</sub>PPtS: C, 49.92; H, 3.44; N, 8.32. Found: C, 49.69; H, 3.04; N, 8.28%. IR (ATR): 3441(w), 3318 (w), 1603, 1556, 1520, 1434, 1304, 1233, 791, 743, 689, 538 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 6.13 (d, 1H, Ar-*H*); 6.65 (br s, 2H, NH<sub>2</sub>); 6.72 (d, 1H, Ar-*H*); 7.10 (t, 1H, Ar-*H*); 7.47-7.55 (m, 9H, Ar-*H*); 7.61 (d, 1H, Ar-*H*); 7.68-7.73 (dd, 6H, Ar-*H*); 7.75-7.77 (d, 1H, Ar-*H*); 8.80 (d, 1H, CHN) ppm. <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 102.75, 117.21, 117.57, 125.86, 127.48, 128.72 (*Ar*); 129.13 (d, *J* [<sup>31</sup>P-<sup>13</sup>C] = 42.04 Hz, *Ar*); 130.14, 130.75 (*Ar*); 132.21 (d, *J* [<sup>31</sup>P-<sup>13</sup>C] = 23 Hz, *Ar*); 135.15 (d, *J* [<sup>31</sup>P-<sup>13</sup>C] = 45.96 Hz, *Ar*), 139.90 (d, *J* [<sup>31</sup>P-<sup>13</sup>C] = 26.84 Hz, *Ar*); 147.70 (CHN); 171.28 (d, *J* [<sup>31</sup>P-<sup>13</sup>C] = 45.96 Hz, CS) ppm.

### X-ray Crystallography

Diffraction data were measured with a Bruker SMART Apex II CCD area-detector diffractometer (graphite-monochromated Mo-*K*α radiation, λ = 0.71073 Å). The orientation matrix, unit-cell refinement, and data reduction were all handled by the Apex2 software (SAINT integration, SADABS absorption correction).<sup>30</sup> The structures were solved using direct or Patterson methods in the program SHELXS-97 and were refined by the full matrix least-squares

method on  $F^2$  with SHELXL-97.<sup>31</sup> All the non-hydrogen atoms were refined anisotropically. The structure of Pd(L)(LH<sub>2</sub>) contains disordered solvent molecules which could not be satisfactorily modeled. The data were therefore treated with the SQUEEZE routine of PLATON.<sup>32</sup> For Drawings of the molecules were produced with XSEED.<sup>33</sup> Crystal data and refinement details are summarized in Tables S4 and S5†.

### Cytotoxicity assay

**Cell culture.** The cell lines were provided by Faculty of Medicine, University of Malaya. All the cells were cultured in either Dulbecco's modified Eagle's medium (DMEM) or RPMI 1640 supplemented with 10% fetal bovine serum (Sigma Aldrich) and L-glutamine at 37 °C in a 5% CO<sub>2</sub> humidified atmosphere.

**MTT cytotoxicity test.** MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was carried out as described by Mosmann,<sup>34</sup> with some modifications. The cells were seeded into 96-well plates (5000 cells/well) and allowed to adhere overnight. Pt(L)PPh<sub>3</sub> was pre-dissolved in dimethyl sulfoxide (DMSO) and diluted to the desired concentrations (eight concentrations, 0.39-50 µg/mL), such that the final concentrations of DMSO did not exceed 0.5%. Each cell line was treated with the test compound solutions (three wells on a plate for each concentration) for 24, 48 and 72 h. Treated and untreated cells were inspected qualitatively using an inverted light microscope (100 X). Then, 10 µl of MTT (5 mg/mL) was added to each well and the plates were incubated at 37 °C for 4 h. The media was then gently aspirated, and 100 µl DMSO was added to dissolve the formazan crystals. The amount of formazan product was measured spectrophotometrically at 570 nm using a microplate reader (Power Wave X 340). The concentration of the test compound required for 50% inhibition of cell growth (IC<sub>50</sub>) was determined by interpolation of regression analysis.

### Apoptosis detection with fluorescence microscopy

**AO/PI staining assay.** The cancer cells were seeded in a 25 mL culture-flask ( $1 \times 10^6$  cells/mL) and treated with the Pt complex at the corresponding  $IC_{50}$  concentrations for 48 h (control cells were treated with 0.5% DMSO vehicle). The cells were then washed with phosphate buffered saline (PBS) and suspended in 500  $\mu$ L of PBS followed by addition of a 1:1 mixture of AO (10  $\mu$ g/ml) and PI (10  $\mu$ g/ml). A drop of the suspension was placed on a glass slide and covered with a cover slip. Images of the cells were taken by a UV-fluorescence microscope within 30 min.

**Annexin V staining.** The cancer cells ( $1 \times 10^6$  cells) were seeded on a chamber slide and were treated with the respective  $IC_{50}$  concentrations of the Pt complex for 48 h (control cells were treated with 0.5% DMSO vehicle). The media was then removed and the slide was washed with PBS. Treated and control cells were exposed to FITC-annexin V for 15 min according to the manufacturer's protocol (BD Pharmingen, USA). After a 488 nm excitation, green fluorescence (emission at 515 nm) was visualized by an inverted fluorescence microscope.

### DNA Fragmentation analysis

The cancer cells were seeded in a 25 mL culture-flask ( $1 \times 10^6$  cells/mL) and treated with the respective  $IC_{50}$  concentrations of the test compound for 48 h (control cells were treated with 0.5% DMSO vehicle). The cells were then washed with PBS. The treated and control cells were washed with PBS and harvested. Cellular DNA was extracted using a Genomic DNA purification kit (Promega, Madison, WI). The DNA fragments were then separated by a 1.5% agarose gel electrophoresis at 50 V for 1.5 h. The Gels were then stained with ethidium bromide and visualized on a UV-illuminator.

## Cell cycle analysis

Cell cycle analysis was carried out by propidium iodide flow cytometry as described elsewhere.<sup>35</sup> Briefly, the cancer cells ( $1 \times 10^6$ ) were seeded in three culture flasks and were treated with the respective  $IC_{50}$  concentrations of the Pt complex for 24 h and 48 h. The cells were then washed with PBS, suspended in 90% ethanol and kept overnight (4°C). The pellets were further washed with PBS and suspended in PBS at 37 °C. 25 µl of RNase A (10 µg/ml) and 50 µl of propidium iodide (10 µg/ml) were added to the mixture and the cells were incubated at 37°C for 30 min. The distribution of the cell cycle was measured by a FACsCANTO II flow cytometer, and the data were analyzed with Prism 5.

## Electronic supplementary information (ESI):

CCDC reference numbers 939908-939913 for  $ZnBr_2(LH_2)_2 \cdot 2MeOH$ ,  $CdCl_2(LH_2)_2 \cdot MeOH$ ,  $Cd(OAc)_2(LH_2)_2 \cdot 2MeOH$ ,  $Pd(L)(LH_2)$ ,  $Pd(L)(PPh_3) \cdot DMF$  and  $Pt(L)(PPh_3) \cdot 0.75 MeOH$ , X-ray crystallographic files in CIF format, tables for the selected geometrical parameters for the crystal structures, tables for crystal data and refinement parameters,  $^1H$ ,  $^{13}C$  NMR, HSQC and HMBC spectra of selected compounds.

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**Text for the table of contents entry**

The Pt(II) complex of a new series of thiosemicarbazone complexes exhibits cancer-selective cytotoxicity



### List of figure captions

**Fig. 1** Various coordination modes observed in metal complexes of thiosemcarbazone ligands (1-8); the chemical diagram of LH<sub>2</sub>

**Scheme 1.** Reactions of LH<sub>2</sub> with the selected metal salts

**Fig. 2** The molecular structure of ZnBr<sub>2</sub>(LH<sub>2</sub>)<sub>2</sub> with thermal ellipsoids drawn at the 30% probability level. Methanol solvate molecules are not shown.

**Fig. 3** The molecular structure of CdCl<sub>2</sub>(LH<sub>2</sub>)<sub>2</sub> with thermal ellipsoids drawn at the 30% probability level. Methanol solvate molecules are not shown.

**Fig. 4** The molecular structure and atom labeling scheme of Cd(OAc)<sub>2</sub>(LH<sub>2</sub>)<sub>2</sub> (25% probability ellipsoids). Methanol solvate molecules are not shown.

**Fig. 5** The molecular structure of Pd(L)(HL<sub>2</sub>) with thermal ellipsoids drawn at 30% probability level.

**Fig. 6** The molecular structure of Pd(L)(PPh<sub>3</sub>) with thermal ellipsoids drawn at 50% probability level. The DMF solvate molecules are not shown.

**Fig. 7** The molecular structure and atom labeling scheme of Pt(L)(PPh<sub>3</sub>) (50% probability ellipsoids). Methanol solvate molecules are not shown.

**Fig. 8** Crystal packing view of Pt(L)(PPh<sub>3</sub>).0.75MeOH, showing channels along the *c*-axis. Hydrogen bonds are depicted as red dashed lines.

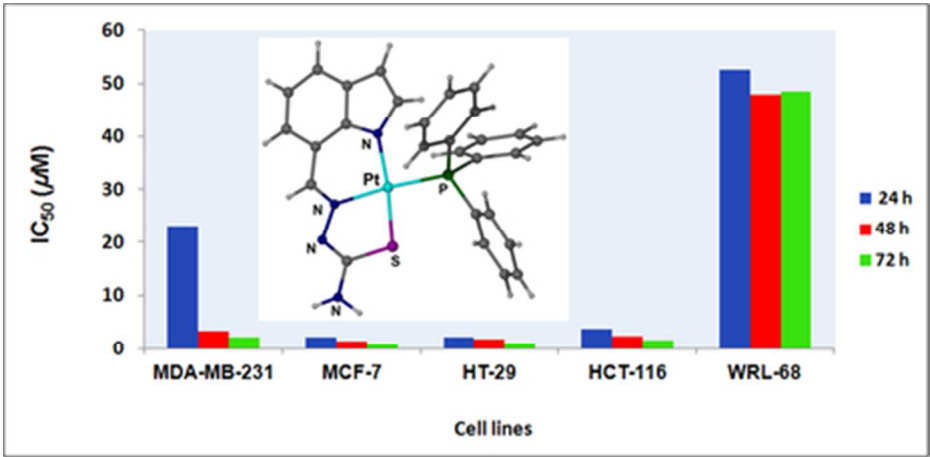
**Fig. 9** Cytotoxicity effect of Pt(L)(PPh<sub>3</sub>) on normal liver WRL-68 cells

**Fig. 10** Fluorescent microscopy of AO/PI stained cancer cells. The first row shows the cells without any treatment and the second row shows the cells after treatment with IC<sub>50</sub> concentrations of Pt(L)PPh<sub>3</sub> for 48 h. Cells with intact membrane are stained green. Apoptotic cells appear in orange-yellow and the necrotic cells are stained red.

**Fig. 11** Fluorescence microscopy of the cancer cells stained by FITC-annexin V. The first row shows the cells without any treatment and the second row shows the cells treated with Pt(L)PPh<sub>3</sub> at IC<sub>50</sub> concentrations for 48 h. FITC-annexin V positive cells show bright green fluorescence.

**Fig. 12** Gel electrophoresis of apoptotic DNA fragmentation. Lane A: 1000 bp molecular weight marker; lane B: untreated MDA-MB-231 cells; lane C: treated MDA-MB-231 cells; lane D: untreated MCF-7 cells; lane E: treated MCF-7 cells; lane F: untreated HT-29 cells; lane G: treated HT-29 cells; lane H: untreated HCT-116 cells; lane I: treated HCT-116 cells.

**Fig. 13** Flow cytometric analysis of cell cycle distribution in the four cancer cell lines treated with  $IC_{50}$  concentrations of  $Pt(L)PPh_3$  for 24 and 48 h. Data were shown as Mean  $\pm$  SEM. \*  $p < 0.05$  vs. controls.



The Pt(II) complex of a new series of thiosemicarbazone complexes exhibits cancer-selective cytotoxicity  
39x19mm (300 x 300 DPI)

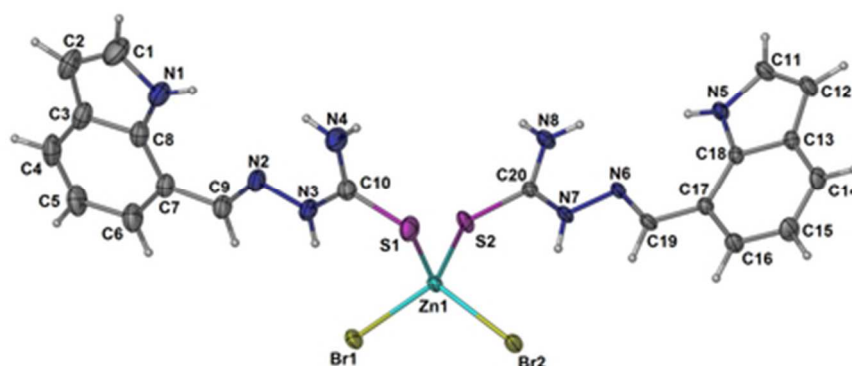


Fig. 2 The molecular structure of  $\text{ZnBr}_2(\text{LH}_2)_2$  with thermal ellipsoids drawn at the 30% probability level. Methanol solvate molecules are not shown.  
36x16mm (300 x 300 DPI)

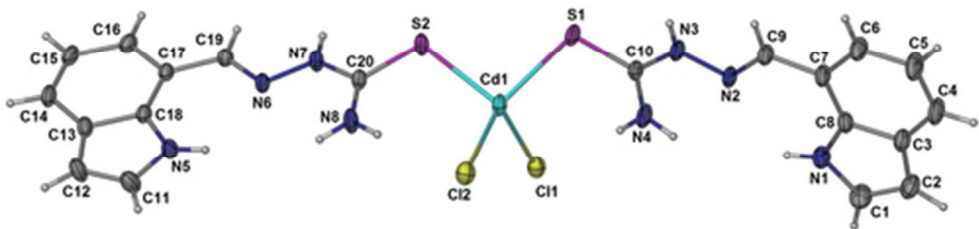


Fig. 3 The molecular structure of  $\text{CdCl}_2(\text{LH}_2)_2$  with thermal ellipsoids drawn at the 30% probability level. Methanol solvate molecules are not shown.  
43x22mm (300 x 300 DPI)

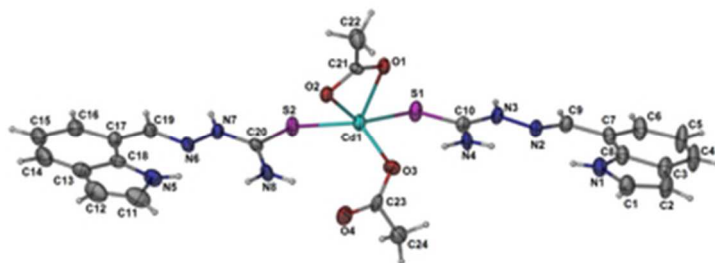


Fig. 4 The molecular structure and atom labeling scheme of  $\text{Cd}(\text{OAc})_2(\text{LH}_2)_2$  (25% probability ellipsoids). Methanol solvate molecules are not shown.  
30x11mm (300 x 300 DPI)

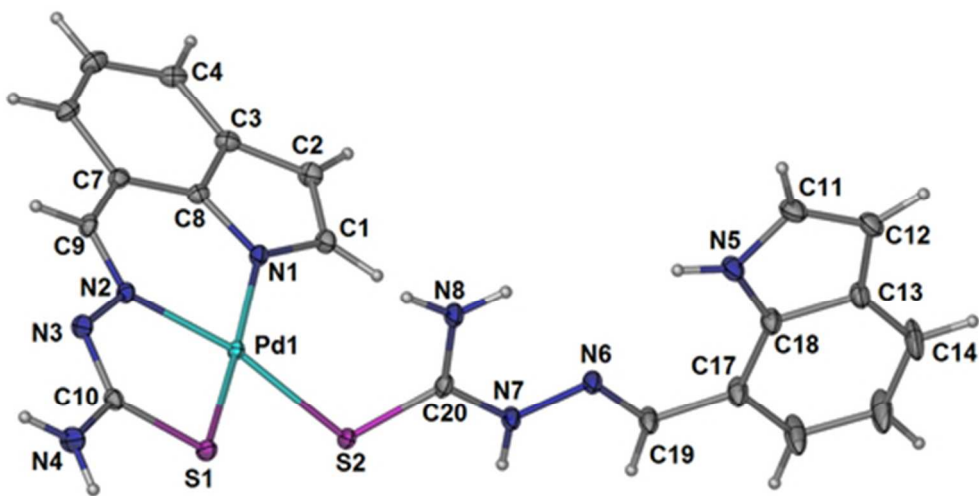


Fig. 5 The molecular structure of Pd(L)(HL2) with thermal ellipsoids drawn at 30% probability level.  
42x21mm (300 x 300 DPI)



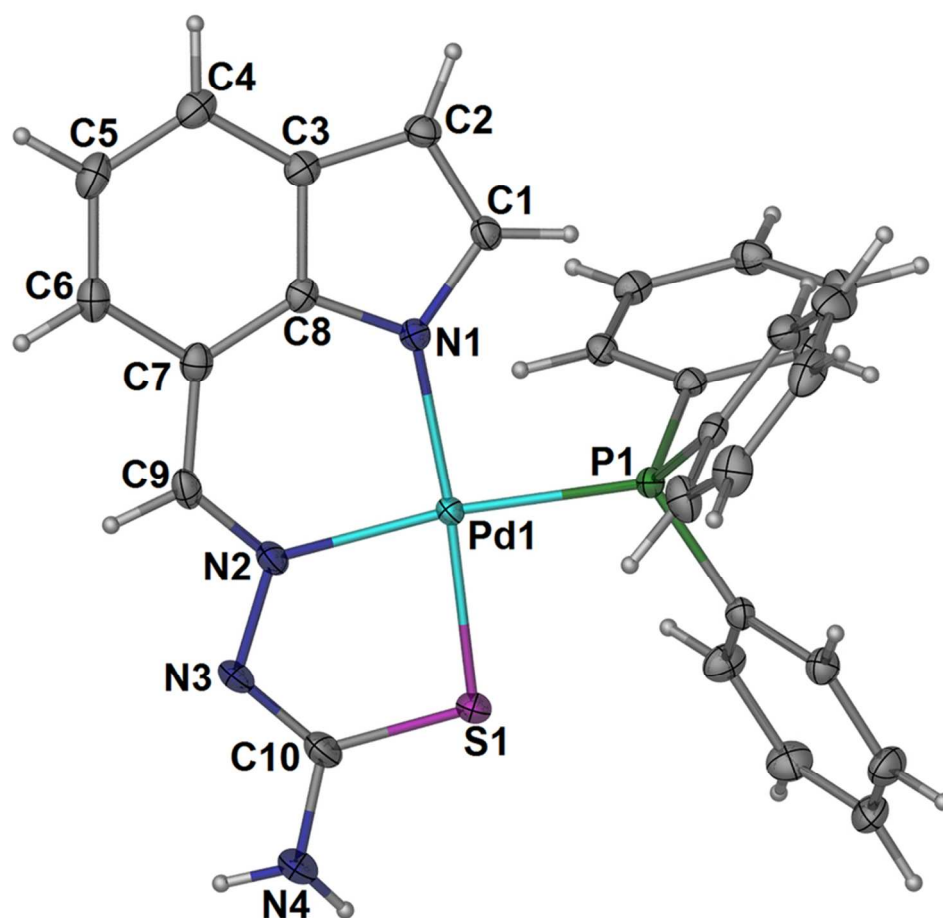


Fig. 6 The molecular structure of Pd(L)(PPh<sub>3</sub>) with thermal ellipsoids drawn at 50% probability level. The DMF solvate molecules are not shown.  
81x79mm (300 x 300 DPI)

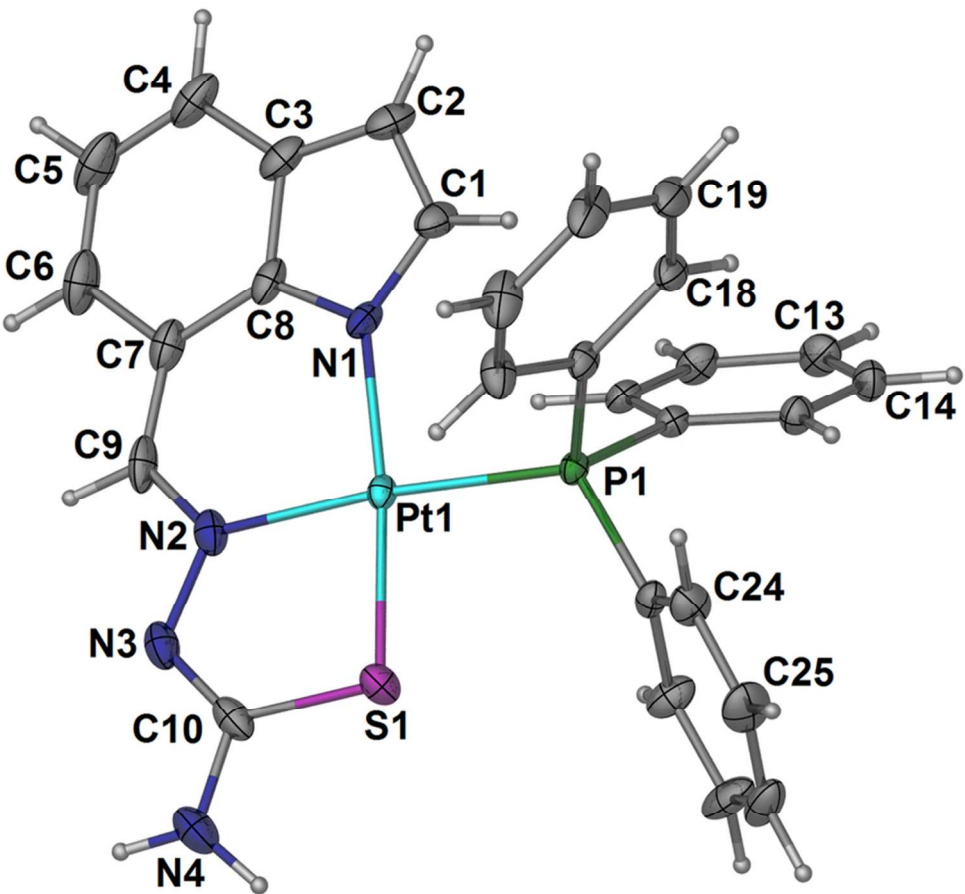


Fig. 7 The molecular structure and atom labeling scheme of Pt(L)(PPh<sub>3</sub>) (50% probability ellipsoids). Methanol solvate molecules are not shown.  
78x73mm (300 x 300 DPI)

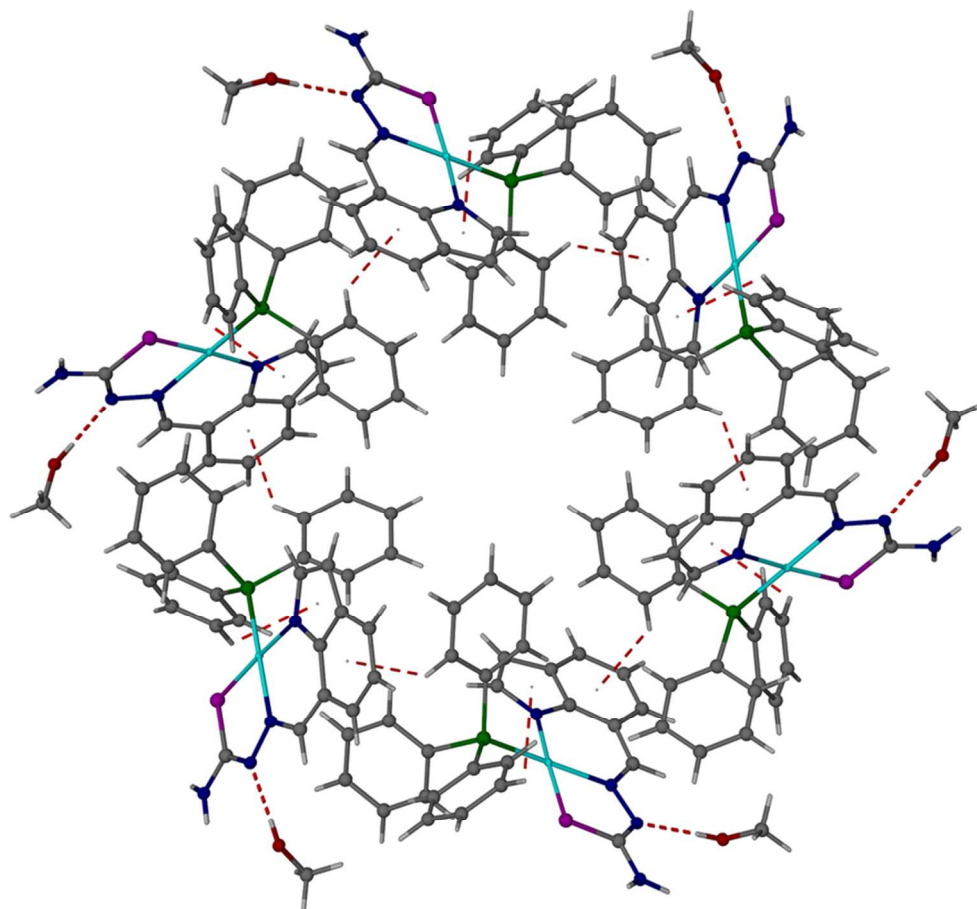


Fig. 8 Crystal packing view of Pt(L)(PPh<sub>3</sub>).0.75MeOH, showing channels along the c-axis. Hydrogen bonds are depicted as red dashed lines.  
76x70mm (300 x 300 DPI)

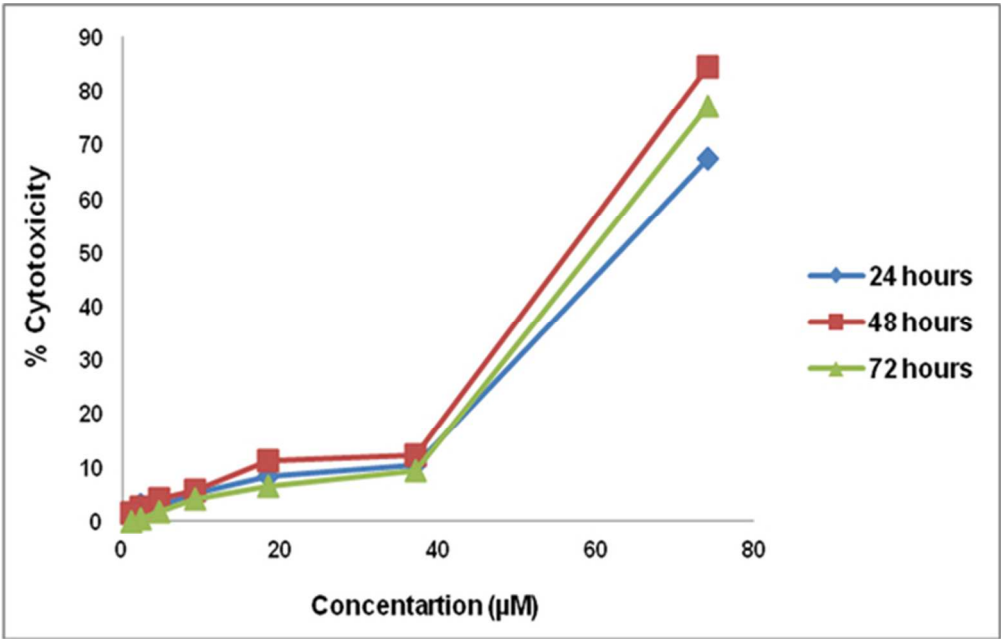


Fig. 9 Cytotoxicity effect of Pt(L)(PPh3) on normal liver WRL-68 cells  
52x33mm (300 x 300 DPI)

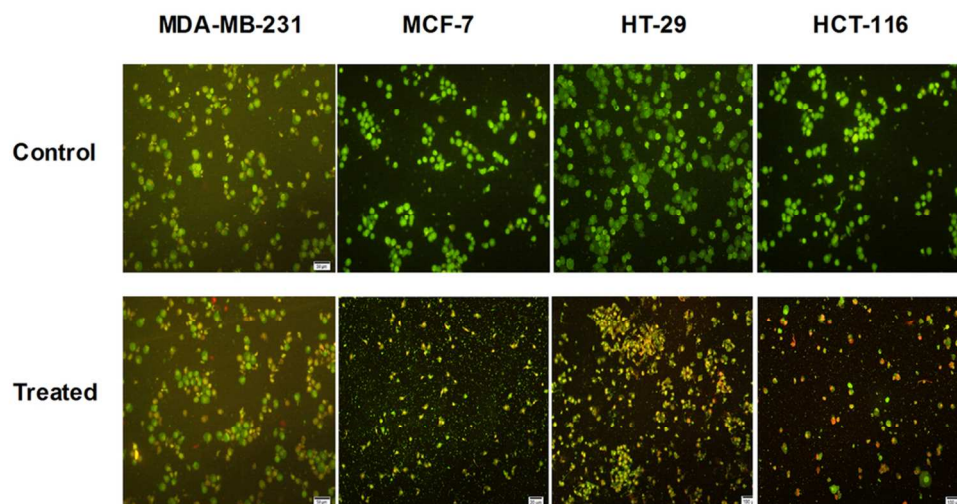


Fig. 10 Fluorescent microscopy of AO/PI stained cancer cells. The first row shows the cells without any treatment and the second row shows the cells after treatment with IC<sub>50</sub> concentrations of Pt(L)PPh<sub>3</sub> for 48 h. Cells with intact membrane are stained green. Apoptotic cells appear in orange-yellow and the necrotic cells are stained red.  
90x48mm (300 x 300 DPI)

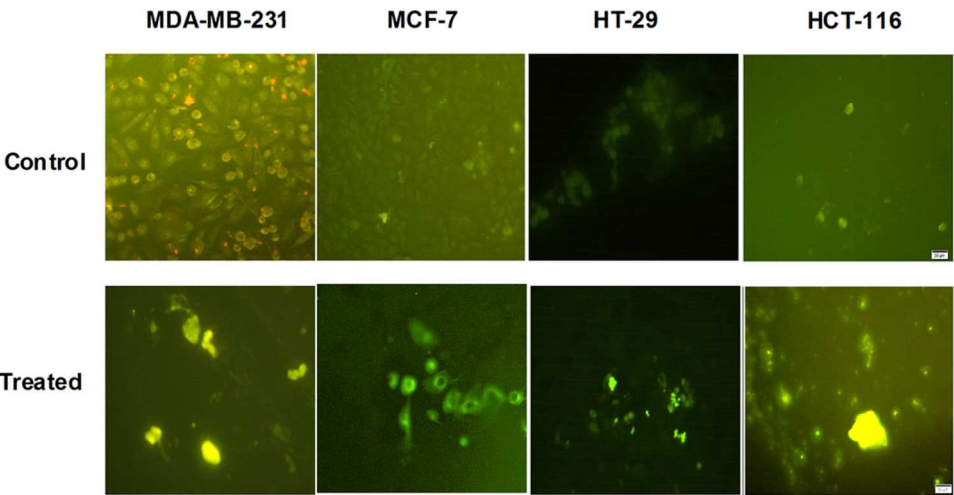


Fig. 11 Fluorescence microscopy of the cancer cells stained by FITC-annexin V. The first row shows the cells without any treatment and the second row shows the cells treated with Pt(L)PPh3 at IC50 concentraions for 48 h. FITC-annexin V positive cells show bright green fluorescence.  
88x46mm (300 x 300 DPI)

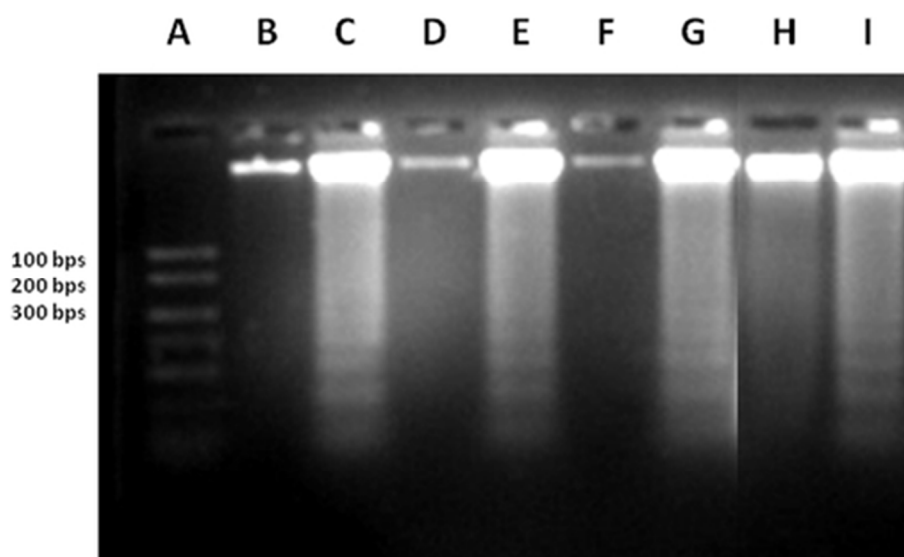


Fig. 12 Gel electrophoresis of apoptotic DNA fragmentation. Lane A: 1000 bp molecular weight marker; lane B: untreated MDA-MB-231 cells; lane C: treated MDA-MB-231 cells; lane D: untreated MCF-7 cells; lane E: treated MCF-7 cells; lane F: untreated HT-29 cells; lane G: treated HT-29 cells; lane H: untreated HCT-116 cells; lane I: treated HCT-116 cells.

47x27mm (300 x 300 DPI)

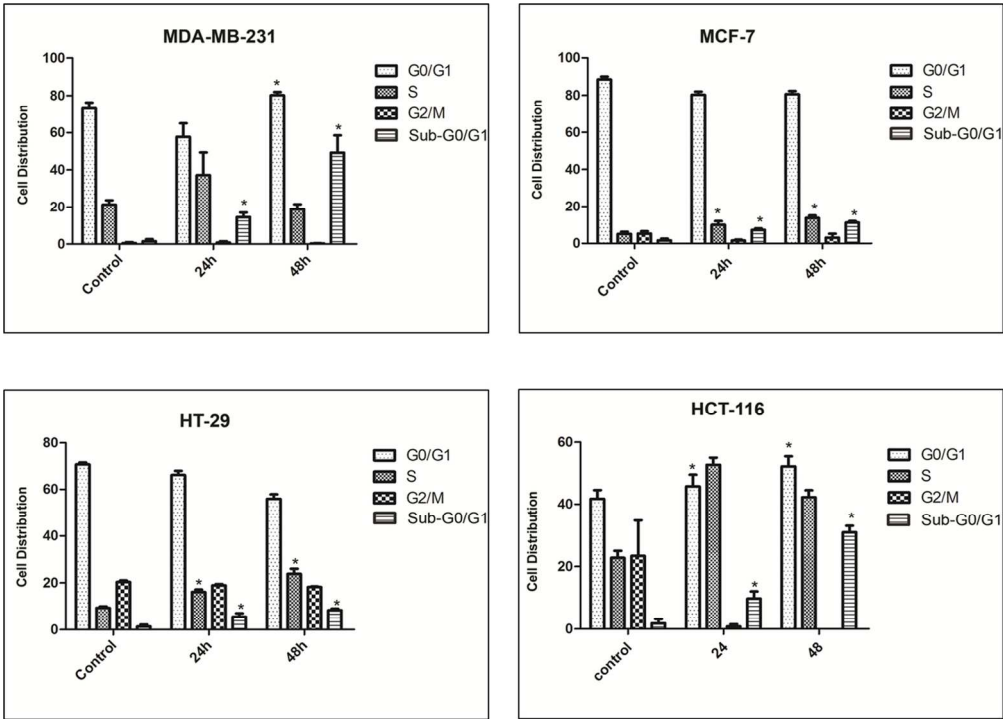


Fig. 13 Flow cytometric analysis of cell cycle distribution in the four cancer cell lines treated with IC50 concentrations of Pt(L)PPh3 for 24 and 48 h. Data were shown as Mean  $\pm$  SEM. \*  $p < 0.05$  vs. controls. 122x87mm (300 x 300 DPI)